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MICROPROPAGATION OF MANGOSTEEN (*Garcinia mangostana*)[1)]

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ABSTRACT

Conventional propagation of mangosteen is still facing some problems, such the limited fruiting season and seed number, and the slow growth of seedling. In vitro *culture is an alternative technique to solve the problems. A study was carried out to obtain a suitable technique for* in vitro *propagation of mangosteen that enable to produce plantlets with high level of shoot multiplications and root formations as well as high level of acclimatized shoots and plantlet growths. Three levels of BA (1, 3, and 5 mg/ml) were used as treatments for shoot inductions and axillary bud multiplications on the MS basal medium. Treatments for root induction were combinations of two basal media (MS and WPM), two concentrations of media (full strength and ¼ strength), and two levels of IBA (5 and 10 mg/l). Root inductions were done* ex situ *by dipping the shoots in IBA solutions (100-200 ppm) for 1-2 hours, followed by planting onto the best acclimatization medium. The acclimatization was done on two different media (soil only and soil + compost) and under two different environments (glasshouse only and incubation room + glasshouse). Results of the experiment showed that the highest percentage of seed growth and number of shoots per seed was obtained on the basal medium containing 5 mg/l BA. The highest number of axillary bud multiplication was obtained on the medium containing 3 mg/l BA. The MS medium + 5 mg/l IBA promoted 75% rooting. The plant acclimatization on soil + compost in the glasshouse with 75% shading promoted the fastest plant growth. During the acclimatization, up to 75% of the shoots treated with dipping in 100 ppm IBA solution for one hour grew well. After 4 months, the roots developed to secondary and tertiary roots.*

[**Keywords**: *Garcinia mangostana,* micropropagation]

INTRODUCTION

Mangosteen is a tropical fruit that has a good prospect as export commodity. Export of mangosteen fruits has been increasing annually. According to Tridjaya (2003), volume of mangosteen export in 1999 reached 4743 tons at a price of US$ 3,887,816 and in 2000 increased to 7182 tons at a price of 5,885,038 that equal to 44% of the total Indonesian fruit export.

Currently, the mangosteen plants found in the production centers are not grown in monoculture, but in a mixture with other trees and mostly old trees. Rejuvenation of the plants has not been fully successful since the plants are growing and fruiting slowly. Plant propagation through seeds is facing several problems. A plant grown from seed will fruit for the first time after 15-17 years (Sarwono 1999). The plant bears fruit only once or twice a year and each fruit has only one or two good seeds. Mangosteen seeds are recalcitrant which will not survive for long, therefore continuous plant propagations by seeds are difficult. Vegetative propagation has not been fully successful besides the plants produced by vegetative propagation grow very slowly, weak, not uniform, and slow flowering (Normah *et al*. 1995; Cruz 2001). Mangosteen that are propagated from approach grafting in the nursery will bear fruits after 6 years. Propagation using this technique, however, needs a large number of branches as a source of plant materials. Propagation by shoot grafting has been done with 48% level of success (Jawal *et al*. 1989).

In vitro propagation is expected to solve the problems of mass propagation of uniform, good performance and continuous annual supply of plant material. Researchers in Malaysia reported that MS medium + BA was enable of yielding best shoot multiplication (Normah *et al*. 1992; Teo 1992). According to Goh *et al*. (1990), the best shoot multiplication was obtained on WPM medium + 5 mg/l BA. Triatminingsih *et al*. (1993) reported that spraying of mangosteen shoot tips (source of explants) with 0.5 ppm BA + 1 ppm GA3 prior to culturing produced the largest number of growing explants (42%), while explants produced from cotyledons were more than that of shoot tips.

Goh *et al*. (1990) reported that addition of IAA to roots yielded lower explant percentage than those treated with 20 mg/l IBA. Pertamawati (1997) reported that MS medium + 15 ppm 2iP + 0.5 ppm IBA produced the highest number of rooting explants. Sinaga (1999) stated that the highest percentage of rooting explants was obtained when the explants were cultured on ½ MS medium + 4 mg/l IBA + 3 mg/l NAA. A study was carried out to develop *in vitro* techniques for propagation of mangosteen that have a

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high level of shoot multiplication and root formation, as well as high percentage of acclimatization level.

MATERIALS AND METHODS

The study was conducted from 2002 to 2004 in the plant tissue culture laboratory of the Indonesian Center for Agricultural Biotechnology and Genetic Resources Research and Development (ICABIOGRAD). Plant material used in the study was mangosteen seed of a local cultivar directly collected from the tree. The seeds were cleaned from their fruit fleshes and surface sterilized by dipping in ethanol 70% for 5 minutes followed by clorox 30% for 10 minutes, and clorox 20% for 5 minutes. The experiments were divided into four trials, i.e. (1) bud/shoot induction from the seed, (2) multiplication of axillary buds, (3) rooting induction and (4) acclimatization.

Bud/Shoot Induction

Each of the surface sterilized seeds was sliced to four pieces and planted onto *in vitro* culture bottle containing the shoot induction medium. The medium consisted of three different media, i.e. MS basic medium + three levels of BA concentration (1, 3 and 5 mg/l). Each treatment consisted of 15 seeds as replications, one seed (four seed pieces) per culture bottle. The parameters observed were percentage of the growing seed pieces, number of buds/shoots per seed, and number of leaves grew from a bud. Percentage of the growing seeds was calculated by counting the number of seed pieces grew divided by the total seed pieces grown. After the shoots reached approximately 3-month old, some explants were subcultured onto the shoot multiplication medium, while some others were directly sub-cultured on the rooting medium.

Multiplication of Axillary Buds

Explants used in this trial are shoots without shoot tips (terminal buds). As in the shoot induction trial, the treatments used are similar, which is by growing the explants onto the MS medium containing three different levels of BA concentration (1, 3 and 5 mg/l). In this case, one culture represented one replication. Observations were made on additions of culture height (cm), leaf number per culture, and branch number per culture. The additions of culture height, leaf number, and branch number per culture were calculated based on the differences between height of cultures, number of leaves, and number of branches after the treatment and prior to the treatment.

Rooting Induction

The trial was done using two different basic media, i.e. MS and WPM; two formula of each medium were used, i.e. ¼ and 1 full strength. Two levels of IBA concentration were used, i.e. 5 and 10 mg/l. Parameters observed were percentage of rooted cultures and root lengths. The percentage of rooted cultures was counted by dividing the number of rooted culture by the total number of cultures. Plantlets obtained from this trial were used in the acclimatization trial.

Acclimatization

During the acclimatization, the plantlets were grown on two different media, i.e. soil only and soil + compost (1:1), and under two different environments (glasshouse only and culture room + glasshouse). Parameters observed were number of leaves and addition of plant heights.

The root induction was also done *ex vitro* using buds that have not been induced its rooting *in vitro*. First, the buds were dipped in 100-200 ppm IBA solutions for 1 or 2 hours then, they were grown on pots containing a mixture of soil + compost (1:1). The parameters observed were percentage of plant survival and plant growth. The dead or survive plant was indicated by colors of the plant, that the plant still survive. The plant growth was indicated by the emergence of two new leaflets with a reddish color. The acclimatized plantlets were sheltered under a plastic shelter for 2 months. The experimental site was 75% shaded using a paranet.

RESULTS AND DISCUSSION

Bud and Axillary Shoot Induction

Results of the bud/shoot induction indicated that a large number of buds from the seeds were growing from the seed section. Most of them, however, in the form of nodules or small shoots (without a developing leaf pair) that made them difficult to count. The counting was therefore done only based on plant shoots that have fully developed leaf pairs. Generally, the higher the BA content in the medium, the higher the percentage of the growing seed sections, the number of shoots per seed section, and the number of leaf pairs per shoot (Table 1). Addition of 5 mg/l BA in the medium gave the highest percentage of seed growth (100% g) with the highest number of shoots per seed was 2.7, and the highest leaf number was 2.9. These results were similar to that of Dewi *et al*. (1999) who mentioned that a BA level of 5 mg/l in the MS ½ N medium (MS + ½ nitrogen content)

induced the growth of shoots from the seeds up to 82.2% on a 7-week-old culture. Teo (1992) also reported that the use of 5 mg/l BA in the medium induced shoot multiplications, but with small shoot sizes and varied morphological performances.

Compared to results of the *in vitro* trial, the direct seed germination in the field generally gives a low level of success. Cruz (2001) reported that the mangosteen seed germination percentages in the field were ranging only from 21 to 83%, depending on the seed freshness and the total number of growing shoots, which is usually only one shoot per seed, rarely more than one shoots per seed

Table 1. Effect of BA concentrations in the medium on percentage of seed growth, shoot number per seed, and leaf number per shoot of mangosteen after 3 months of seeding.

Concentration of BA (mg/l)	Seed growth (%)	Average number of shoots/ seed	Average number of leaves/ shoot
1	40.0	1.1	1.4
3	66.7	1.7	2.8
5	100.0	2.7	2.9

Figure 1. Steps in the *in vitro* propagation of mangosteen; A = plant shoots growing from four seed sections; B = plant shoots emerging from ¼ of seed piece, C = an axillary shoot on the shoot multiplication medium; D = root growth on the rooting medium; E = plantlet ready for acclimatization; F = seedling on the acclimatization medium composed of soil + compost (1:1); G = 4 month-old mangosteen seedlings, and H = mangosteen rootlets consisting of primary, secondary and tertiary roots.

(10%), that is on seeds that have polyembrionic embryos (seeds that have more than one embryos). Nevertheless, not all of the growing shoots *in vitro* may be subcultured in the root induction media because of the small size of the shoots (< 1 cm high). In general, the number of shoots that directly transferable onto the subculture medium were 3-5 shoots per seed, while the other shoots need to be grown onto the growth medium. The shoot performances growing from the seed that was cut into four pieces are shown in Figure 1A, while the shoot growth from one the ¼ seed section after subculturing are shown in Figure 1B. The cut shoots transferred onto the subculture medium may be in the form of axillary shoots (Figure 1C). At the level of 3 mg/l BA, the multiplication rate was the highest on parameters of height increase, leaf number, and branch number (Table 2). Goh *et al*. (1988) reported that 31% of the cut shoots that were subcultured onto medium containing 1 mg/l BA produced axillary shoots on its nodes.

Rooting Induction

Results of the rooting trial showed that the basic MS medium was unable to induce root growth of mangosteen. Diluting the medium into ¼ MS dilution induced root growth, particularly when combined with the addition of 5 mg/l IBA. TheWPM medium and its dilution also induced root growth (Table 3). When another trial was done on treatments that give high yields, it was shown that the ¼ MS medium + 5 mg/l IBA was the best treatment with 75% rooting percentage (Table 4). In this case the higher level of N in the MS medium than that in the WPM medium may cause retardation of root induction on mangosteen culture, because the growth was dominated by the shoots. Similarly, when the basic medium was diluted (its micronutrients) the root growth induced more. Besides the medium dilution, the increase of IBA contents in the medium was also capable of inducing root growth, although this was not

Table 2. Growth increases of mangosteen cultures after 3 months of subculture on the shoot multiplication media containing three different concentrations of BA.

Concentration of BA (mg/l)	Increase in culture height (cm)	Increase in leaf number per culture	Branch number per culture
1	0.2 ± 0.2	1.1 ± 1.1	0.8 ± 0.8
3	0.2 ± 0.1	1.1 ± 1.1	0.8 ± 0.8
5	0.1 ± 0.1	1.4 ± 1.4	0.5 ± 0.5

Numbers in each column are averages of 6-9 replications, including their standard deviations.

Table 3. Effect of basal media (MS and WPM) and IBA concentrations on percentages of rooting explants and average numbers of root length of mangosteen at 6 months after subculture.

Treatment	Rooting explant (%)	Root length (cm)
MS + IBA 5 mg/l	0	0
MS + IBA 10 mg/l	0	0
¼ MS + IBA 5 mg/l	50	2.6 ± 0.6
¼ MS + IBA 10 mg/l	0	0
WPM + IBA 5 mg/l	50	0.6 ± 0.6
WPM + IBA 10 mg/l	33.3	1.8 ± 1.8
¼ WPM + IBA 5 mg/l	33.3	0.9 ± 0.9
¼ WPM + IBA 10 mg/l	66.7	1.9 ± 1.9

Numbers under root length represent average from 3-4 replications and their standard deviations.

Table 4. Effect of three different growth media on growth of mangosten roots at 4 months after subculture.

Treatment	Rooting explant (%)	Root length (cm)
WPM + IBA 5 mg/l	37.5	0.6 ± 0.6
¼ WPM + IBA 10 mg/l	37.0	1.3 ± 1.3
¼ MS + IBA 5 mg/l	75.0	1.6 ± 1.6

Numbers in each column are averages of 8 replications and for root length were followed with their standard deviations.

occur when the addition of IBA was done to the full MS medium. A similar result was reported by Goh *et al*. (1988). The MS medium containing 5 mg/l IBA induced mangosteen root growth from a 2 week-old culture with low rooting percentage (7%). Later, Goh *et al*. (1994) reported that the use of WPM medium containing 0.1 mM IBA induced root growth of 6 week-old mangosteen culture to 80%.

Visually, the root produced from the *in vitro* culture was a single root without branching and root hairs on plantlets in the culture bottle and ready for acclimatization (Figures 1D and 1E). Goh *et al*. (1990) reported similar results and mentioned that the rooting system was similar to that rooting system of the naturally grown seed. This indicates that both *in vitro* and *ex vitro* cultures of mangosteen have rooting systems that do not develop well, so that the nutrient uptakes were inhibited. Thus, the seedling growth was retarded (Table 1 dan 2) which is shown by the low number of leaves and branches.

Acclimatization

Results of the acclimatization trial showed that all the planting materials which produced roots in the rooting medium, survived and grew on the acclimatization medium. The acclimatization process may be done directly in the glasshouse without first preplanting in the culture chamber. The soil + compost medium (1:1) gave a better growth to the plants than soil only. The best results from treatment by growing the plants in the soil + compost medium in the glasshouse were shown by the increases in leaf numbers and plant heights by 2.2 and 1.5 cm, respectively (Table 5, Figure 1F). Root inductions might also be done *ex vitro* at the acclimatization step using planting materials that were dipped in a suspension of IBA without first doing root growth induction *in vitro*. Root induction *in vitro* by dipping the planting materials in the IBA solution was also conducted by Te-chato and Lim (1999) and Triatminingsih *et al*. (2001).

Observation after 2 months of acclimatization indicated that the size of planting material and the length of dipping period in the 100 ppm IBA solution affected the success of acclimatization. The best result was obtained by using big size of plant material (> 2 cm) and dipping in the IBA solution for one hour (Table 6). The most important parameter was the growth percentage. The growth was indicated by the emergence of new shoots with a pair of red leaves. The green color of the tissue gives no assurance of the plant survival. The high level of percentage of green tissue on

Table 5. Increase in leaf numbers and plant heights of mangosteen after acclimatization, 2 months after planting.

Treatment	Increase in leaf number	Increase in plant height (cm)
Glasshouse		
Soil	1.7 ± 0.8	0.7 ± 0.4
Soil + compost (1:1)	2.2 ± 0.4	1.5 ± 1.1
Culture room		
Soil	1.3 ± 1.0	0.5 ± 0.5
Soil + compost (1:1)	1.3 ± 1.1	0.9 ± 0.5

Numbers in each column are averages of 8 replications followed with their standard deviations.

Table 6. Percentages of acclimatization success *ex vitro* of mangosteen based on the growing plants from three different sizes of planting material treated with combinations of dipping time and IBA concentrations, 2 months after planting.

Size of planting material	Plant performance (%) after dipping the planting material in					
	100 ppm IBA for one hour		100 ppm IBA for 2 hours		200 ppm IBA for one hour	
	Green	Growing	Green	Growing	Green	Growing
Big (> 2 cm)	75	75	50	25	50	50
Medium (1-2 cm)	80	40	80	60	80	40
Small (< 1 cm)	83	50	90	50	90	40

those which come from smaller size of planting material might be due to the low level of transpiration. The planting material that fail to grow within 2 months, although the tissue color is still green, will gradually die within the following month. The planting material that produced shoots will then produced roots. According to Pertamawati (2003), young leaves or shoots synthesize auxin that induces root growths. The roots then synthesize cytokinin that functions inducing the shoot growth. Four months afterward, seedlings from the *in vitro* culture have had more than five leaf pairs (Figure 1G). The shoot growth was under a compatible condition with the root growth. When the plant was taken of from the growth medium without rootlets, it showed better growth as indicated by the growth of secondary and tertiary rootlets (Figure 1H).

CONCLUSION

Mangosteen plant may be propagated through *in vitro* culture. The MS medium + 5 mg BA/l induced shoot growth of up to 100% with the largest number of shoots and leaves. The best multiplication medium for the planting material was MS medium + 3 mg BA/l. Root growth induction on the ¼ MS medium + 5 mg IBA/l increased percentage of the rooting culture by 75%. The best medium for plant acclimatization in the glasshouse and under 75% shading is a mixture of soil and compost (1:1). The planting material that has been treated to induce its root growth *ex vitro* by dipping in a 100 ppm IBA solution for one hour showed 75% growth after the acclimatization.

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